J. Parasitol., 96(1), 2010, pp. 228–229

Seroprevalence of Canine Leishmaniasis and American Trypanosomiasis in Dogs From Grenada, West Indies

Alexa C. Rosypal, Shanesha Tripp, Christopher Kinlaw, R. N. Sharma*, D. Stone*, and J. P. Dubey†, Department of Natural Sciences and Mathematics, College of Science, Technology, Engineering and Mathematics, Johnson C. Smith University, 100 Beatties Ford Road, Charlotte, North Carolina, 28216; *Pathobiology Academic Program, School of Veterinary Medicine, St. George's University, Grenada, West Indies; †United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Animal Parasitic Diseases Laboratory, Building 1001, Beltsville, Maryland 20705-2350. *e-mail: acrosypal@jcsu.edu*

ABSTRACT: Canine leishmaniasis and American trypanosomiasis (AT) are caused by related hemoflagellated parasites, *Leishmania* spp. and *Trypanosoma cruzi*, which share several common host species. Dogs are reservoirs for human infections by both pathogens. We determined the prevalence of antibodies to *Leishmania spp.* and *T. cruzi* in dogs from Grenada, West Indies. We examined 70 dog sera using the qualitative immunochromatographic dipstick tests (ICTs) based on recombinant antigens specific for visceral leishmaniasis and AT. Antibodies to visceral *Leishmania* were not detected in Grenadian dogs by ICT. Using the canine dipsticks for AT, antibodies to *T. cruzi* were determined in 3 (4.3%) of the 70 dogs. Results from this study indicate that dogs in Grenada are exposed in low levels to *T. cruzi*, but not to visceral *Leishmania* spp. at all.

Leishmania spp. and *Trypanosoma cruzi* are closely related zoonotic hemoflagellates that can cause fatal infections in humans and dogs. Both *Leishmania* spp. and *T. cruzi* have been described in various domestic and wild mammals. Domestic canines play an important role in the epidemiology of both visceral leishmaniasis (VL) and American trypanosomiasis (AT) because they are important reservoir hosts for human infections with their respective causative parasites (Chappuis et al., 2007; Rosypal, Cortes-Vecino et al., 2007). Worldwide there are approximately 12 million people with leishmaniasis (Desjeux, 2004), and the Centers for Disease Control and Prevention (CDC) (http://www.cdc.gov/chagas/epi.html. Accessed 1/11/2010) estimates that up to 11 million people are currently infected with *T. cruzi* (WHO, 2007).

Leishmaniasis and AT are endemic in most of Latin America, but limited information is available about them specifically in the Caribbean. The Caribbean comprises a chain of islands, also called the West Indies, from Cuba to Trinidad. Grenada is a southern island near the northern coast of Venezuela. Little is known of *Leishmania* spp. and *T. cruzi* infections in dogs in Grenada, West Indies. In the present report, we examined the serological prevalence of *Leishmania* spp. and *T. cruzi* in dogs from Grenada, West Indies, using commercial immunochromatographic dipstick tests for canine VL and AT.

Canine samples for this study were obtained from various sources in Grenada, West Indies. Tested animals included 67 dogs from St. George's University (SGU) School of Veterinary Medicine for routine spay or neuter, dogs participating in free SGU vaccination clinics conducted in several parishes, those from the Grenada Society for the Prevention of Cruelty to Animals, dogs from parishes throughout Grenada identified by word of mouth, and some stray dogs. A signed owner consent form was obtained for all pet animals. Prior to sampling, all dogs were examined by a veterinarian and given a body condition score. Any dog exhibiting clinical signs of systemic disease, or if a blood sample could not be obtained, was excluded from the study.

As part of a *Toxplasma gondii* and *Neospora caninum* characterization study (Dubey et al., 2008), blood was collected from jugular, cephalic, or lateral saphenous vein. Serum was separated by centrifugation and stored frozen (−20 C) at SGU until all samples were obtained. Frozen sera were sent by overnight airmail to the United States Department of Agriculture Animal Parasitic Diseases Laboratory, Beltsville, Maryland. Frozen sera were subsequently sent to the Department of Natural Sciences and Mathematics, College of Science, Technology, Engineering and Mathematics, Johnson C. Smith University, Charlotte, North Carolina, where serology for antibodies to *Leishmania* spp. and *T. cruzi* was performed.

DOI: 10.1645/GE-2238.1

Commercial canine immunochromatographic (ICT) dipstick assays were conducted for qualitative antibody detection to visceral *Leishmania* spp. and *T. cruzi*. Antibody testing was performed according to the manufacturer's test procedure. The ICT assays are based on recombinant antigens and have been developed into a dipstick format. The tests are a proprietary gold mix based on recombinant antigens. Previous reports have demonstrated their superior performance over traditional serological screening tests based on crude antigens or whole organisms (Houghton et al., 2000; Scalone et al., 2002). Canine sera were tested for antibodies to recombinant K39 (rK39) (Kalazar Detect™ Canine Rapid Test, InBios International Ltd., Seattle, Washington), which is an amastigote protein specific to visceral *Leishmania* spp., and it does not cross-react with antibodies to *T. cruzi* (Burns et al., 1993). Anti-*T. cruzi* antibodies were evaluated by canine ICT (Trypanosoma Detect™ MRA Rapid Test; Inbios International Ltd., Seattle, Washington) based on multiepitope recombinant antigens.

Sera were tested by ICT according to the manufacturer's test procedure. Briefly, dog serum (20 µl) was aliquotted onto a test strip. The dipstick then was placed in a well of a 96-well round bottom tissue culture plate and provided chase buffer (100–150 µl). Test results were read after 10 min. According to the test procedure, after 10 min, a red control line and a second line in the test field appeared if the result was positive. The presence of a red band only on the control line indicated a negative result.

Antibodies to visceral *Leishmania* spp. were not found in Grenadian dogs. Seropositivity for *T. cruzi* was detected in 3 dogs (4.3%). Based on a previous study, the dogs exhibiting antibodies to *T. cruzi* were seropositive for neither *N. caninum* nor *T. gondii*.

The ICT dipsticks used in this study are based on recombinant antigens and are considered ideal diagnostic tools for testing in remote areas (Chappuis et al., 2007). The ICT is a qualitative assay, but it detects canine antibodies to *Leishmania* or *T. cruzi* with titers as low as 1:50 or 1:25, respectively (Rosypal et al., 2005; Rosypal, Tidwell, and Lindsay, 2007). In addition, several studies using the recombinant antigens in the ICT demonstrated high specificity compared to traditional serological assays (Burns et al., 1993; Rosypal et al., 2005; Rosypal, Tidwell, and Lindsay, 2007).

In the present study, none of the dogs tested positive for antibodies to visceral *Leishmania* parasites by the ICT. Human leishmaniasis has been occasionally documented from several of the Caribbean islands (Grimaldi et al., 1989; Zeledón, 1992; Ashford, 2000), but to our knowledge no cases of human or canine leishmaniasis have been documented from the island of Grenada. Zoonotic cutaneous and visceral leishmaniasis in the Latin American and Caribbean region primarily involves transmission between humans and dogs or other sylvatic reservoirs (reviewed by Hotez et al., 2008). Cutaneous leishmaniasis has been described in the Dominican Republic, Martinique, Guadeloupe, Trinidad, and the French Antilles (Grimaldi et al., 1989; Zeledón, 1992). In 1966 the first indigenous case of visceral leishmaniasis from the Caribbean islands occurred on Guadeloupe in a 7-yr-old girl (Zeledón, 1992) and has subsequently been described in Martinique (reviewed by Grimaldi et al., 1989).

Although few cases of leishmaniasis have been reported from the region, several species of sand fly vectors are dispersed throughout the Caribbean islands (reviewed by Zeledón, 1992). An animal reservoir for leishmaniasis has not been identified in the Caribbean, but the black rat (*Rattus rattus*) is suspected (Zeledón, 1992). The prevalence of leishmaniasis in Grenada is unknown, and whether dogs can be a source of infection for humans on this island is not clear.

The seroprevalence of *T. cruzi* in dogs from Grenada was 4.3% (3 of 67 dogs) in the current work. The ICT is a qualitative assay, so titers were not

determined. False positive cross-reactions with the *Leishmania* spp. ICT were not observed in *T. cruzi*–positive dogs, which supports the specificity of the visceral leishmaniasis and AT ICT serological assays.

Trypanosoma cruzi is an arthropod-borne parasite of medical and veterinary importance. AT is a significant public health concern and causes serious cardiac disease in both humans and dogs. Canine AT is marked by myocardial dysfunction in both the acute and chronic phases of disease (Barr, 2006). In Latin America, domestic dogs are considered a major reservoir in the domestic transmission cycle of *T. cruzi* (Gürtler et al., 2007), and they can serve as sentinels of human infections (Estrada-Franco et al., 2006).

Thirteen countries in Latin America and the Caribbean (LAC), are endemic for AT (reviewed by Hotez et al., 2008); however, current data on the burden and distribution of *T. cruzi* infections do not exist for most of this region (Reithinger et al., 2009). Triatomine vectors tend to live in cracks and crevices in poor-quality housing, and infected dogs can increase the risk of domiciliary transmission (Gürtler et al., 2007). Efforts of the Southern Cone Initiative to eliminate AT have significantly reduced new human infections with *T. cruzi* (Moncayo and Ortiz Yanine, 2006); however, the disease remains endemic in many parts of Latin America. Animal reservoirs and human immigrants into nonendemic areas could result in the emergence or reemergence of AT (Estrada-Franco et al., 2006).

In the LAC region, several countries close to Grenada are affected by AT. *Trypanosoma cruzi* levels vary depending on location and host species. In 1965 the first presumed cases of *T. cruzi* infection were described in Trinidad in humans with symptoms of cardiomyopathy (Anonymous, 1965); *T. cruzi*–like parasites were found in Caribbean insect vectors (Anonymous, 1965). Authochthonous acute AT has been confirmed in Suriname (Oostburg et al., 2003), and a survey of 1,487 people in French Guiana revealed 0.5% had detectable *T. cruzi* IgG serum antibodies (Aznar et al., 2004). Recently in Venezuela, seroprevalence has been reported at 1.6% in humans (Rojas et al., 2008). Antibody levels in Venezeulan dogs range from 6.4% (Rojas et al., 2008) to 67.6% (Crisante et al., 2006), depending on the serological test used and location within the country. To our knowledge, there are no published reports of canine AT in Grenada, West Indies.

Protozoan parasites that are structurally similar can differ antigenically (Dubey et al., 2008). *Leishmania* spp. and *T. cruzi* are closely related parasites that use different insect vectors to infect mammalian hosts. The overall lack of canine antibodies to viscerotropic *Leishmania* spp. and low *T. cruzi* seroprevalence (4.3%) could reflect antigenic distinction between these 2 organisms or a difference in distribution of the vector species on the island of Grenada. In the present study, we describe antibodies to *T. cruzi* for the first time in dogs from Grenada, West Indies. Because of the public health importance of this parasite, further epidemiological research should be conducted to determine the presence of *T. cruzi* in Grenada and to determine the role of dogs as a domestic reservoir on this Caribbean island.

This work was supported by a minigrant to A.C.R. from the Historically Black Universities and Colleges-Undergraduate Program (HBCU-UP) at Johnson C. Smith University, Charlotte, North Carolina.

LITERATURE CITED

Anonymous. 1965. Medical research in the British Caribbean. British Medical Journal **1**(5446): 1372–1373.

Ashford, R. W. 2000. The leishmaniasis as emerging and reemerging zoonoses. International Journal for Parasitology **30:** 1269–1281.

Aznar, C., G. La Ruche, S. Laventure, B. Carme, P. Liegeard, and M. Hontebeyrie. 2004. Seroprevalence of *Trypanosoma cruzi* infection in French Guiana. Memórias do Instituto Oswaldo Cruz **99:** 805–808.

Barr, S. C. 2006. Trypanosomiasis. *In* Infectious diseases of the dog and cat, C. E. Greene (ed.). W. B. Saunders, Philadelphia, Pennsylvania, p. 676–681.

Burns, Jr., J. M., W. G. Shreffler, D. R. Benson, H. W. Ghalib, R. Badaro, and S. G. Seed. 1993. Molecular characterization of a kinesin-related antigen of *Leishmania chagasi* that detects specific antibody in African and American visceral leishmaniasis. Proceedings of the National Academy of Sciences USA **90:** 775–779.

Chappuis, F., S. Sundar, A. Hailu, H. Ghalib, S. Rijal, R. W. Peeling, J. Alvar, and M. Boelaert. 2007. Visceral leishmaniasis: What are the needs for diagnosis, treatment and control? Nature Reviews Microbiology **5:** S7–S16.

Crisante, G., A. Rojas, M. M. Teixeira, and N. Añez. 2006. Infected dogs as a risk factor in the transmission of human *Trypanosoma cruzi* infection in western Venezuela. Acta Tropica **98:** 247–254.

Desjeux, P. 2004. Leishmaniasis: Current situation and new perspectives. Comparative Immunology, Microbiology and Infectious Diseases **27:** 305–318.

Dubey, J. P., D. Stone, O. C. H. Kwok, and R. N. Sharma. 2008. *Toxoplasma gondii* and *Neospora caninum* antibodies in dogs from Grenada, West Indies. Journal of Parasitology **94:** 750–751.

Estrada-Franco, J. G., V. Bhatia, H. Diaz-Albiter, L. Ochoa-Garcia, A. Barbabosa, J. C. Vasquez-Chagoyan, M. A. M. Martinez-Perez, C. Guzman-Bracho, and N. Garg. 2006. Human *Trypanosoma cruzi* infection and seropositivity in dogs, Mexico. Emerging Infectious Diseases **12:** 624–630.

Grimaldi, Jr., G., R. B. Tesh, and D. Mcmahon-Pratt. 1989. A review of the geographic distribution and epidemiology of leishmaniasis in the New World. American Journal of Tropical Medicine and Hygiene **41:** 687–725.

Gürtler, R. E., M. C. Cecerre, M. A. Lauricella, M. V. Cardinal, U. Kitron, and J. E. Cohen. 2007. Domestic dogs and cats as sources of *Trypanosoma cruzi* infection in rural northwestern Argentina. Parasitology **134:** 69–82.

Hotez, P. J., M. E. Bottazi, C. Franco-Paredes, S. K. Ault, and M. R. Periago. 2008. The neglected tropical diseases of Latin America and the Caribbean: A review of disease burden and distribution and a roadmap for control and elimination. PLoS Neglected Tropical Diseases **9:** e300 (1–11).

Houghton, R. L., D. R. Benson, L. D. Reynolds, P. D. McNeill, P. R. Sleath, M. J. Lodes, Y. A. Skeiky, R. Badaro, A. U. Krettli, and S. G. Reed. 2000. Multiepitope synthetic peptide and recombinant protein for the detection of antibodies to *Trypanosoma cruzi* in patients with treated or untreated Chagas' disease. Journal of Infectious Diseases **181:** 325–330.

Moncayo, A., and M. I. Ortiz Yanine. 2006. An update on Chagas disease (human American trypanosomiasis). Annals of Tropical Medicine and Parasitology **100:** 663–677.

Oostburg, B. F., J. E. Anijs, G. P. Oehlers, H. O. Hiwat, and S. M. Burke-Hermelin. 2003. Case report: The first parasitologically confirmed autochthonous case of acute Chagas disease in Suriname. Transactions of the Royal Society of Tropical Medicine and Hygiene **97:** 166–167.

Reithinger, R., R. L. Tarleton, J. A. Urbina, U. Kitron, and R. E. Gürtler. 2009. Eliminating Chagas disease: Challenges and a roadmap. British Medical Journal **338:** 1044–1046.

Rojas, M. E., P. Várquez, M. F. Villarreal, C. Velandia, L. Vergana, Y. H. Morán-Borges, J. Ontiveros, M. Yelitza Calderón, M.A. Chiurillo-Siervo, C. Rodríguez-Bonfante Cdel, et al. 2008. An entomological and seroepidemiological study of Chagas' disease in an area in central-western Venezuela infested with *Triatoma maculata* (Erichson 1848). Cadernos de Saúde Pública **24:** 2323–2333.

Rosypal, A. C., J. A. Cortés-Vecino, S. M. Gennari, J. P. Dubey, R. R. Tidwell, and D. S. Lindsay. 2007. Serological survey of *Leishmania infantum* and *Trypanosoma cruzi* in dogs from urban areas of Brazil and Colombia. Veterinary Parasitology **149:** 172–177.

———, R. R. Tidwell, and D. S. Lindsay. 2007. Prevalence of antibodies to *Leishmania infantum* and *Trypanosoma cruzi* in wild canids from South Carolina. Journal of Parasitology **93:** 955–957.

———, G. C. Troy, R. B. Duncan, Jr., A. M. Zajac, and D. S. Lindsay. 2005. Utility of diagnostic tests used in diagnosis of infection in dogs experimentally inoculated with a North American isolate of *Leishmania infantum infantum*. Journal of Veterinary Internal Medicine **19:** 802–809.

Scalone, A., R. Deluna, G. Oliva, L. Baldi, G. Satta, G. Vesco, W. Mignone, C. Turilli, R. Mondesire, D. Simpson, et al. 2002. Evaluation of the *Leishmania* recombinant K39 antigen as a diagnostic marker for canine leishmaniasis and validation of a standardized enzyme-linked immunosorbent assay. Veterinary Parasitology **104:** 275–285.

World Health Organization (WHO). 2007. Available at http://www.who.int/ctd/chagas/disease.htm.

Zeledón, R. 1992. Leishmaniasis in the Caribbean islands. Annals of the New York Academy of Science **653:** 154–160.